

In vitro anti-diabetic activity of flavonoids and pheophytins from *Allophylus cominia* Sw . on PTP1B, DPPIV, alpha-glucosidase and alpha-amylase enzymes

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Abstract

Background

Ethno-botanical information from diabetic patients in Cuba led to the identification of *Allophylus cominia* as a possible source of new drugs for the treatment of type 2 diabetes mellitus (T2-DM).

Experimental

Chemical characterization of the extracts from *A. cominia* was carried out using chromatographic and spectroscopic methods. The extracts were tested for their activity on PTP1B, DPPIV, α-glucosidase enzymes and α-amylase.

Results

The flavonoid rich fractions from *A. cominia* inhibited DPPIV enzyme (75.3±2.33%) at 30 µg/ml and produced a concentration-dependent inhibition against DPPIV with a Ki value of 2.6 µg/ml. At 30 µg/ml, flavonoids and pheophytins extracts significantly inhibited PTP1B enzyme (100±2.6% and 68±1% respectively). The flavonoids, pheophytin A and pheophytin B fractions showed significant concentration-dependent inhibition against PTP1B with Ki values of 3 µg/ml, 0.64 µg/ml and 0.88 µg/ml respectively. At 30 µg/ml, the flavonoid fraction significantly inhibited α-glucosidase enzyme (86±0.3%) in a concentration-dependent pattern with a Ki value of 2 µg/ml. None of the fractions showed significant effects on α-amylase. Fatty acids, tannins, pheophytins A and B, and a mixture of flavonoids were detected in the methanolic extract from *A. cominia*. The identified flavonoids were mearnsitrin, quercitrin, quercetin-3-alloside, and naringenin-7-glucoside.

Conclusion

The pharmacological effects of the extracts from *A. cominia* earlier observed in experimental diabetic models was confirmed in this study. Thus a new drug or formulation for the treatment of T2-DM could be developed from *A. cominia*.

Abbreviations: PTP1B, (Protein Tyrosine Phosphatase 1B); DPPIV, (Dipeptidyl peptidase-4); Ki, (inhibitory constant); TFMS (, [Bis(4-trifluoromethylsulfonamidophenyl)–1,4-diisopropylbenzine), P32/98 ((3N-((2-((3N-[(2S,6,3-3S)–2-amino-3-methyl-pentanoyl]–1,3-thiazolidine) hemifumarate); T2-DM, type 2 diabetes mellitus)

Keywords: *Allophylus cominia*; DPPIV; PTP1B; alpha-glucosidaseAlpha-glucosidase; andAnd α-amylase

1 Introduction

Type 2 diabetes Mellitus (T2-DM) is a well-known metabolic disease (Alonso-Castro et al., 2008) and it has been considered as one of the most severe chronic diseases. The number of patients suffering from the disease is estimated to be 180 million worldwide and this number is expected to double by 2030 (WHO, 2005).

Allophylus cominia (L.) Sw. (palo de caja) (*A.cominia*) is a plant used in Cuba traditionally for the treatment of T2-DM. Its ethnomedical use was reported in the volume Medicinal, aromatic and poisonous plants of Cuba by the acknowledged botany Juan Tomás Roig y Mesa: “In traditional medicine branches, leaves and logs are used. Formerly used only as a home remedy in baths and cooking against pushes and other intestinal disorders; but since a few years it has acquired great esteem as a remedy against diabetes, with many people among them professionals who have assured us that it is very effective against this disease, being verified immediately after its use the decrease of sugar in the urine (Roig, 2012).

A. cominia is a wild tree, characteristic of the lower arboreal layer of semi-deciduous forests, is very common throughout the island, inhabits mainly in areas of few elevations in the west of the country and grows on the slopes of the hills and river banks, it is indifferent to the soil and persistent in secondary vegetation. It has also been found in Mexico, Jamaica, Haiti and Santo Domingo. It presents alternate leaves 5- folioladas and polygamous flowers, in branched clusters, densely pubescent; 4-sepals, 4-petals and 8-stamens. The fruit is commonly formed by a carpel monosperm, orange or red 4 mm long, subglobose and almost dry. It has not been cultivated massively in Cuba or, as is known, in another part of the world, and when it has been done for the establishment of small plots, cases in which seeds have been used as propagating material and following conventional methods of management. It must respond positively to the propagation by grafts (Roig, 2012).

The plant species *Allophylus cominia* (L.) Sw. (palo de caja), also known as *Rhus cominia* L and *Schmidelia cominia* Sw, is one of the most famous Cuban medicinal plants. *Allophylus cominia* (L.) Sw. is an accepted name of a species in the genus *Allophylus* (family Sapindaceae). Is a native plant which grows as a small shrub up to 9 m, very common on rugged terrain and hillsides all over the Cuban island.

The mechanism of action is still unknown (Sanchez (Sánchez)-et al., 2014). Enzymes such as PTP1B, DPPIV, α-glucosidase and α-amylase have been reported as new drug targets for the treatment of T2-DM and obesity. Inhibitors of these enzymes have been produced using synthesis and natural products isolation (Chen et al., 2009; Na et al., 2006a, 2006b)). Metabolic mechanisms that have a high impact on glucose uptake are numerous, such as activation of glucose transporters GLUT4 in cell plasma membranes, insulin mimetic acting at post-receptor level, activation of AMP Kinase, an increase in nitric oxide, glucose-6P, glycogen stores (Wiernsperger, 2005) and deficiency in PTP1B. Further, several plant extracts may inhibit the action of PTP1B that regulates negatively insulin signalling by dephosphorylating phosphotyrosine residues on insulin receptors. Prolonged DPPIV inhibition has a potential effect on reducing post-prandial glucose levels and HbA1c levels, which confirms that DPPIV inhibitors are novel, efficient and tolerable treatments for T2-DM (Ahrén, 2005). Inhibitors such as acarbose which is an α-amylase and α-glucosidase inhibitor, acts by blocking the activity of these enzymes leading to a reduction in the breakdown of polysaccharides, and thereby decreasing the post-prandial increase in the blood glucose level in addition inducing weight loss (Zhong et al., 2006). The aim of this study is to identify and purify the active compounds in *A. cominia* leaves using phytochemical techniques and then evaluate the inhibitory effect of these extracts on PTP1B, DPPIV, α-amylase and α-glucosidase enzymes and study the kinetics of their inhibition in each of these enzyme assays (Veliz (Véliz) -et al., 2003; Marrero and Capasso, 2007). However Sánchez -et al. et al. in 2014 have separated extracts from *A.cominia* using flash chromatography technique and the fractions tested on PTP1B and DPPIV enzymes showed significant inhibition.

2 Materials and Methods

2.1 PTP1B enzyme assay

Extracts of *A. cominia* at 30 µg/ml were incubated according to the method described by Montalibet et al. (2005) with Protein Tyrosine Phosphatase 1B (PTP 1B, purchased from Invitrogen, Life Technologies, UK) at concentrations of 2 nM for 30 min at 37 °C in a humidified atmosphere containing 5% CO₂. 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, 10 µM, Invitrogen D6567, Molecular Probes, Life Technologies, UK) was then added and incubated for 10 min at 37 °C. TFMS [Bis (4-trifluoromethylsulfonamidophenyl)–1,4-diisopropylbenzene, Calbiochem 540211, 10 mg, Millipore, UK] was used as a standard inhibitor for the PTP1B inhibition assay in a concentration range of 0.0003–3.00 µM. The hydrolysis of DiFMUP by PTP1B enzyme was measured at λ max 355 nm (extinction) and 460 nm (emission).

2.2 DPPIV enzyme assay

Extracts of *A. cominia* at 30 µg/ml were incubated with DPPIV enzyme (purchased from Sigma Aldrich, UK, D7052) at 0.1 mg/ml for 30 min at 37 °C in a humidified atmosphere containing 5% CO₂ (Cooper and Woods, 2009). Glyco-Pro7-amido-4-methylcoumarin hydrobromide (10 µM, Sigma G2761, 25 mg) was then added and incubated for 30 min at 37 °C. P32/98 {(3N-[(2 S, 3 S)–2-amino-3-methyl-pentanoyl]–1,3-thiazolidine) hemifumarate, from Tocris 2136, 10 mg} was used as a standard inhibitor of DPPIV enzyme, a concentration range of 0.0003–3.00 µM was used. The hydrolysis of substrate by DPPIV enzyme was measured at λ max 355 nm (extinction) and 460 nm (emission).

2.3 Alpha-glucosidase enzyme assay

Extracts of *A. cominia* at 30 µg/ml were incubated with α-glucosidase (yeast α-glucosidase EC 3.2.1.20, purchased from Sigma G0660) enzyme (Gulati et al., 2012) at 75 unit/ml for 10 min at 37 °C in a humidified atmosphere containing 5% CO₂. 4-nitrophenyl-glucopyranoside (4 mM, Sigma N1377, 1 g) was then added and incubated for 10 min at 37 °C. Glucosidase (purchased from Sigma, A8980, 1 g) was used as a standard in the assay. The hydrolysis of the substrate by α-glucosidase enzyme was measured at λ max 405 nm.

2.4 Alpha-amylase enzyme assay

Extracts of *A. cominia* at 30 µg/ml were incubated with α-amylase (α-amylase (3.2.1.1) from porcine pancreas, purchased from Sigma A6255) enzyme (Gulati et al., 2012) at 20 µl for 30 min at 37 °C in a humidified atmosphere containing 5% CO₂. 4-nitrophenyl-α-D-maltohexaside (purchased from Sigma 73681 (100 mg)) was then added and incubated for 30 min at 37 °C. Acarbose was used as a standard in the assay system (purchased from Sigma A8980). The hydrolysis of the substrate by α-amylase enzyme was measured at 450 nm.

2.5 Plant material and Phytochemical studies

Allophyllus cominia (L) Sw (Sapindaceae) leaves were collected from forest of Cotilla, San José de las Lajas, Mayabeque, Cuba which is a place previously studied at CENSA, Cuba for the micro localization of the plant. *Allophyllus cominia* (L) Sw (Sapindaceae) is not an endemic plant of Cuba, it is native and well recognized by expertized botany institutions so taxonomically authenticated by Toledo 77922(HAJB) at National Botany Garden and by Professor Fernando Franco Flores in the Laboratory of Botany at the Agriculture University of Havana, Cuba and a voucher specimen of the plant is kept for reference HFA (1769) in the Herbarium of this institution.

The plant samples were dried at 40-40 °C in an oven for 8 hours and subsequently kept for 72 hours in an incubation chamber at 37-37 °C until completely dried. The dry material was milled in a knife mill to a particle size of 3-5 mm. Subsequently, the ground material was stored in polyethylene bags in a dry place at an average temperature of 25 °C and relative humidity of 50%.

Two different extraction methods were carried out: maceration with hexane, ethyl acetate and then methanol. Then the residue of the plant material (from the maceration) was used for hot extraction by soxhlet using the same solvents. First, the plant material (582.0 g) was extracted using three litres by n-hexane, ethyl acetate and methanol. Plant material was left in each solvent for three days.

After each extraction, the solvent was filtrated through Whatman™ filter paper and filtrates were evaporated at 40 °C under vacuum using a rotary evaporator (Büchi Rotavapour) until all solvents were removed. Then the extracts were transferred into small vials using small amounts of the same solvent used for extraction and left under a fume hood at room temperature to obtain solvent-free extracts. TLC and NMR spectroscopies of the three different crude extracts were carried out.

After maceration, the solid from the *A. cominia* leaves left from the filtration of the methanol extract was totally dried under fume hood and then was extracted using the Soxhlet apparatus using n-hexane, ethyl acetate and methanol.

Around 250.0 g (total weight of dry plant material after maceration was 500.0 g) of the plant material was placed in the extraction chamber. About 3.50 L of solvent was used for the extraction. Each extraction was left for two to three days.

The extracts were filtered using filter papers and the filtrates were evaporated at 40 °C under vacuum using a rotary evaporator. The extracts were then transferred into small vials using a small amount of the same solvent of extraction and left under the hood to obtain solvent-free extracts. TLC and NMR spectroscopies of the different crude extracts were carried out.

The hexane and ethyl acetate extracts were combined based on TLC similarity and evaporated under vacuum to obtain the crude extract HEC. This was subjected to silica gel column chromatography and 175 fractions were collected at 10 ml per vial. The fractions were examined by TLC and combined fractions were further analysed by ¹HNMR (Fig. 1). Combined fraction 45-90 was examined by mass spectrometry and by further NMR experiments such as ¹³C, COSY, HMBC and HSQC to determine the structure of the constituents. The methanol extract was subjected to vacuum liquid chromatography (VLC) using gradient elution to obtain a number of sub-fractions. Sub-fractions K, L, M and N eluted by 80:20, 70:30, 60:40 and 50:50 (v/v) ethyl acetate in methanol mixtures were combined. This was then subjected to Sephadex column chromatography eluting with methanol and collecting 5 ml/vial to obtain 190 fractions. Sub-fraction 91-175 re-chromatographed using Sephadex to obtain 92 fractions. Only sub-fraction 66-72 gave a mixture of flavonoids free of fatty acids and tannins. The flavonoids in this flavonoid rich fraction was separated by HPLC using a C-18 amino (IBL-SIL, NH₂, 240×4.60 mm purchased from Phenominex®) - injection volume was 100 µl. The flavonoids were detected at 340 nm and elution was isocratic using 80:20 (v/v) water in acetonitrile. The flow rate was 1 ml/min. Sub-fraction D from the VLC of the methanol extract eluted by 1:1 hexane in ethyl acetate was further purified using silica gel (Silica gel 60, mesh size 20-200 µm from Merck, Germany). About 150 g of silica gel and a solvent system comprising of 70:30 (v/v) n-hexane in ethyl acetate was used and fractions of 1 ml/vial were collected. After TLC using the same solvent system, fractions from 5 to 13 were pooled together and identified by ¹HNMR as well as fractions from 14 to 44. The scheme of separation and isolation of the compounds from the extracts of *A. cominia* is given in Fig. 1.

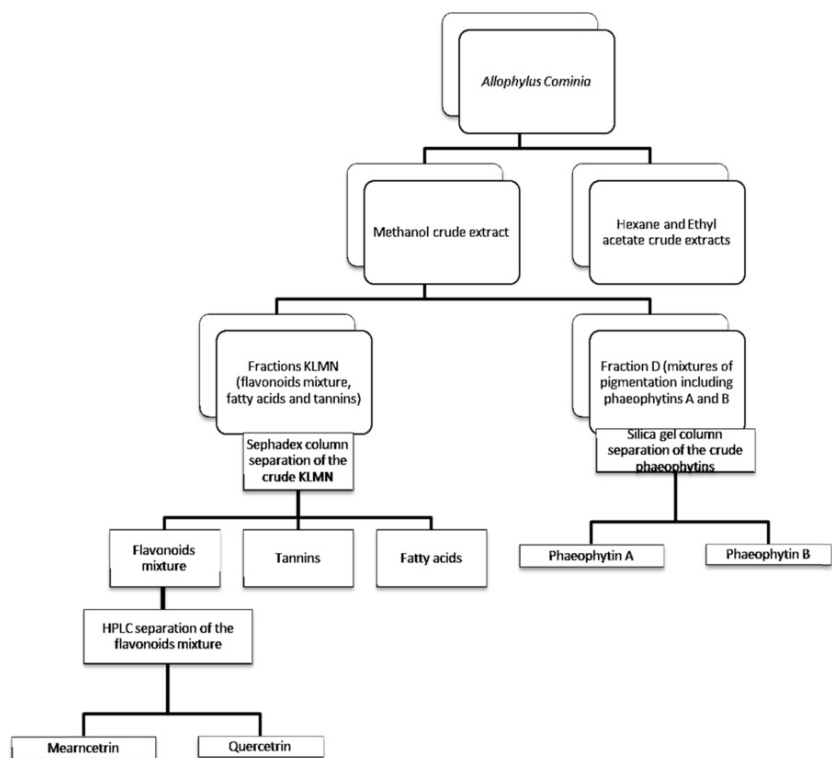


Fig. 1 Fractionation scheme for *Allophylus cominia*.

alt-text: Fig. 4.1

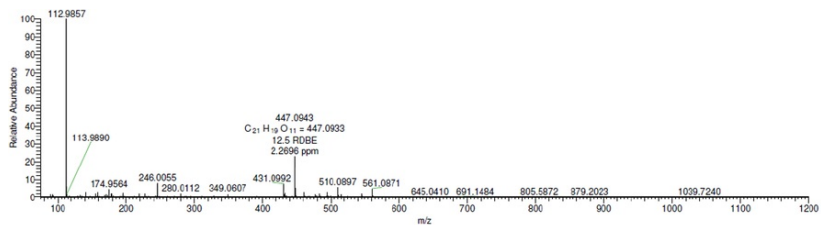
2.6 Statistical analyses

Data was expressed as mean±SEM. Graphs were generated using GraphPad Prism version 4 software. The data were analysed statistically by one way ANOVA and Dunnett Post-test. The level of statistically significance was set at $P<0.05$.

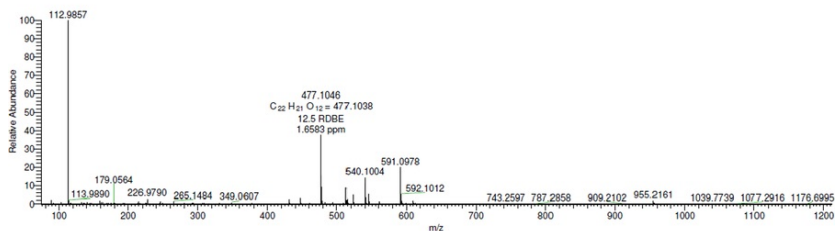
3 Results

3.1 Phytochemical separation and identification of pheophytins and flavonoids in *A. cominia*

Separation of the flavonoids, quercitrin and mearnsitrin, was achieved by HPLC on an amino column, mearnsitrin was collected at 8 min and quercitrin was collected at 10 min. The identification of these two flavonoids was by LC-MS using a C-18 column as shown in Fig. 2 (mearnsitrin MW 447, $C_{21}H_{20}O_{11}$ in Fig. 2A and quercitrin MW 477, $C_{22}H_{22}O_{12}$ in Fig. 2B). The presence of pheophytin A and B was confirmed by comparing the 1H NMR spectral data with literature values for these compounds (Hui et al., 2012; and Atsutoshi et al., 2007). In addition, ^{13}C and HMBC spectra were used to confirm their structures.



A. Quercitrin



B. Meansitrin

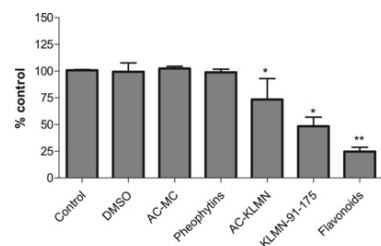
Fig. 2 LC-MS spectrum showing the molecular ions for quercitrin (A) and meansitrin (B).

alt-text: Fig. 2.2

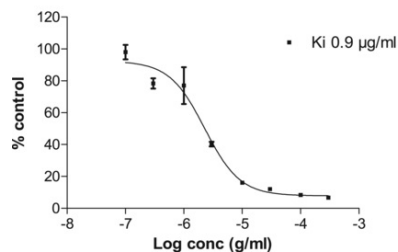
3.2 Enzymes assay

3.2.1 DPPIV assay

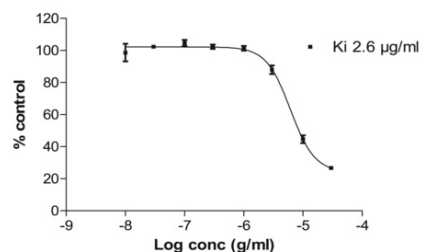
At 30 µg/ml the flavonoid rich fractions (highest concentration tested for the *A. cominia* fractions) significantly ($P < 0.05$) inhibited DPPIV enzyme ($75.3 \pm 2.33\%$) in comparison to the DMSO control (Fig. 3. A). Flavonoids produced a concentration dependent inhibition of DPPIV with a K_i value of 2.6 µg/ml (Fig. 3. C) in comparison to the commercially available inhibitor (P32/98) with K_i value of 0.9 µg/ml (Fig. 3. B). The separated quercitrin and meansitrin did not affect the activity of DPPIV enzyme.



A. Effect of extracts from *A. cominia* on DPPIV enzyme



B. Effect of P32/98 on DPPIV enzyme



C. Effect of flavonoids from *A. cominia* on DPPIV enzyme

Fig. 3 The flavonoid rich fraction from *A. cominia* produced inhibition of DPPIV enzyme in the presence of Gly-Pro substrate. Data represents mean±SEM of DPPIV enzyme hydrolysis (% control) of three independent experiments. Insert shows the effect of different concentrations of P32/98 standard (0.03-100 µM) on DPPIV enzyme in the presence of Glyo-Pro. The data was analysed by Dunnnett post-test. *P value<0.05 versus control. A. Effect of extracts from *A. cominia* on DPPIV enzyme B. Effect of P32/98 on DPPIV enzyme C. Effect of flavonoids from *A. comini* on DPPIV enzyme.

alt-text: Fig. 3

3.2.2 PTP1B assay

Flavonoids and pheophytins extracts from *A. cominia* exhibited potent inhibitory effects on PTP1B enzyme. At 30 µg/ml, the flavonoid rich fractions from *A. cominia* significantly inhibited PTP1B enzyme (100% inhibition) (Fig. 4. B). The flavonoids extract produced a concentration dependent inhibition with a Ki value of 3 µg/ml (Fig. 4. C). Extracts separated from the sub-fraction D significantly inhibited PTP1B enzyme (more than 60% inhibition). At 30 µg/ml, pheophytins A and B inhibited PTP1B enzyme by 65±2% and 57±2% respectively (Fig. 4. D). Pheophytins A and B produced concentration dependent inhibition of PTP1B with Ki values respectively 0.64 µg/ml (Fig. 4. E) and 0.88 µg/ml (Fig. 4. F). Ki values of both pheophytins were lower than that of the commercial available inhibitor (TFMS) with a Ki value of 1.1 µg/ml (Fig. 4. A).

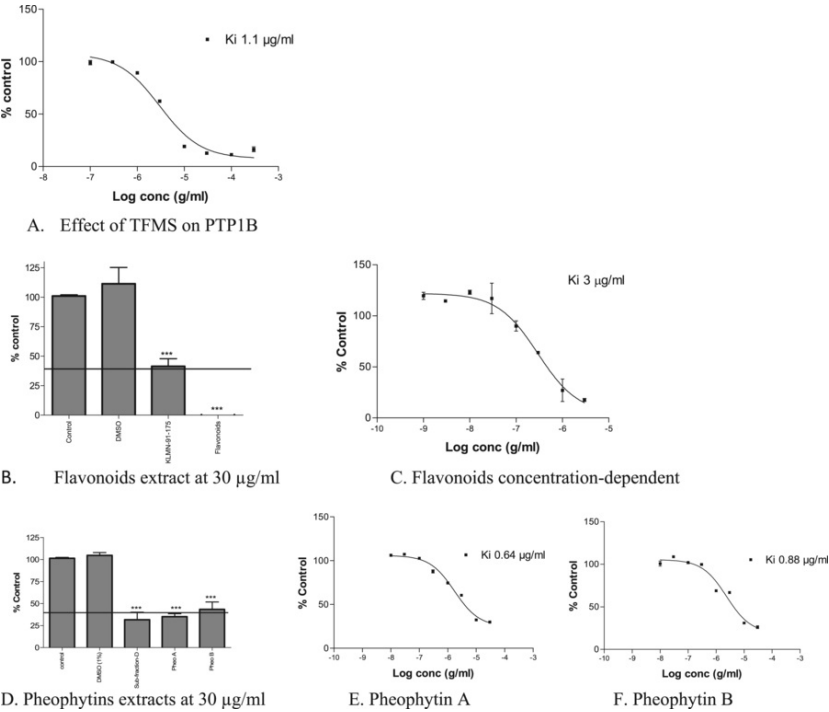


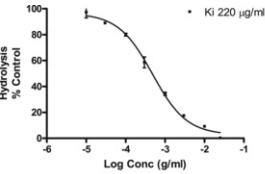
Fig. 4 Flavonoids and pheophytins from *A. cominia* produce inhibition of PTP1B enzyme in the presence of DiFMUP substrate. Data represents mean±SEM of PTP1B enzyme hydrolysis (% control) of three independent experiments. (A) Shows the effect of different concentrations of TFMS standard (0.03-100 µM) on PTP1B enzyme in the presence of DiFMUP. DMSO was used as control. *P value<0.05 versus control. A. Effect of TFMS on PTP1B B. Flavonoids extract at 30 µg/mlC. Flavonoids concentration-dependent D. Pheophytins extracts at 30 µg/ml E. Pheophytin A F. Pheophytin B.

alt-text: Fig. 4

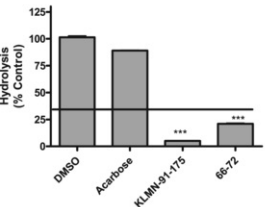
3.2.3 α-Glucosidase assay

The methanolic crude extract and the flavonoid fraction (sample 66-72) from *A. cominia* at 30 µg/ml (Fig. 5. B) produced 95% and 86±0.3% inhibition (14% of the control) respectively. The flavonoid fraction produced a concentration dependent inhibition of α-glucosidase enzyme with a Ki value of 2 µg/ml (Fig. 5. C). Fraction 46-59 that was identified as a mixture of flavonoids, fatty acids or tannic acids also inhibited (94±0.3%) α-glucosidase (Fig. 5. B). By comparison to acarbose (Ki 220 µg/ml), the inhibition by the flavonoids (2 µg/ml) was about 110 fold more potent than that of the commercial available inhibitor (Acarbose). Sub-fraction D (containing pheophytins A and B; 30 µg/ml) also inhibited the α-glucosidase enzyme (46±5.18%, Fig. 5. D)

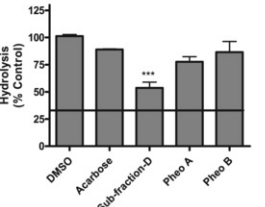
Fig. 5 with a Ki of 9 µg/ml (Fig. 5. E). Ki of the pheophytins extract was also lower than that of Acarbose.



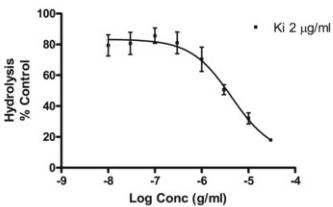
A. Effect of acarbose on α-glucosidase



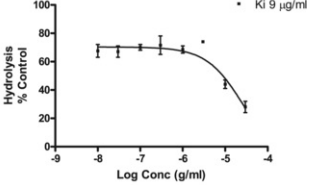
B. Flavonoids extracts at 30 µg/ml



D. Pheophytins extracts at 30 µg/ml



C. Effect of flavonoids at different concentration



E. Concentration dependent effect of pheophytins

Fig. 5 Flavonoids and pheophytins from *A. cominia* produce inhibition of α-glucosidase enzyme in the presence of 4-nitrophenyl-glucopyranoside Data represents mean±SEM of α-glucosidase enzyme hydrolysis (% control) of three experiments. *P value<0.05 versus control.A. Effect o acarbose on α-glucosidase B. Flavonoids extracts at 30 µg/mlC. Effect of flavonoids at different concentration D. Pheophytins extracts at 30 µg/mlE. Concentration dependent effect of pheophytins.

alt-text: Fig. 5.

3.2.4 α-Amylase assay

Acarbose produced a concentration-dependent inhibition of α-amylase enzyme with a Ki value of 0.29±0.05 mg/ml. All extract fractions of *A. cominia* (Fig. 6) were tested on α-amylase enzyme at 30 µg/ml. DMSO (1%) did not produce inhibition of α-enzyme in comparison to the control. Only AC-MC-G, K, LMN-91-175 (flavonoids), AC-HEC-53 (tannins), AC-MC-D-25-44 (pheophytin B) and F1 sample (mearnsitrin) produced statistically significant inhibition of α-amylase, although the inhibition was less than 60% (29%, 10%, 18%, 14% and 9% respectively). However, none of the extracts was considered to be active on α-amylase enzyme.

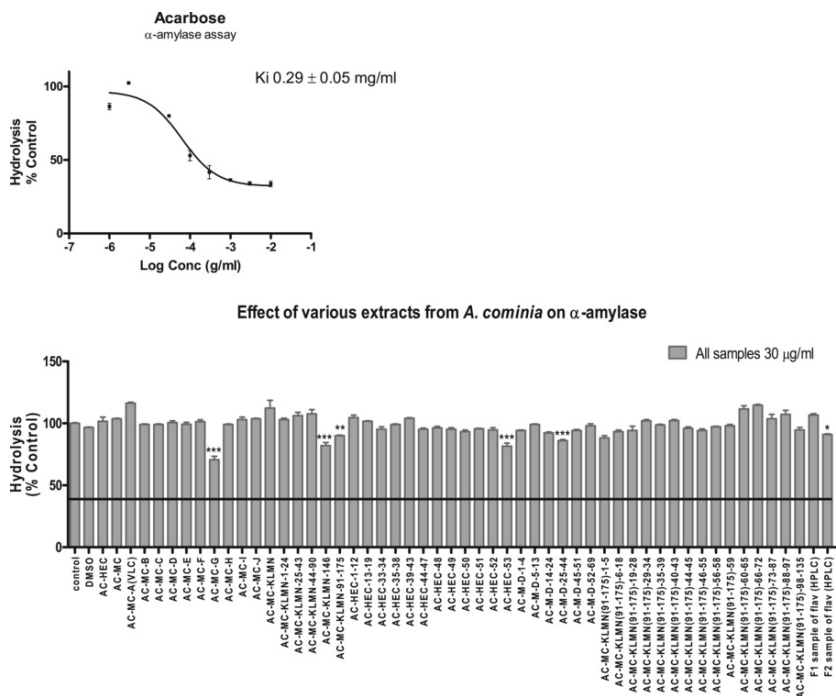


Fig. 6 Effect of *A. cominia* fractions on α -amylase enzyme in the presence of 4-nitrophenyl- α -D-maltohexaside. Data represent mean \pm SEM of enzyme hydrolysis. The insert shows the effect of various concentrations of acarbose standard (10 μ M - 30 mM) on α -amylase enzyme in the presence of 4-nitrophenyl- α -D-maltohexaside. ****P* value <0.05 versus control.

alt-text: Fig. 6

4 Discussion and Conclusion

The ability of DPPIV inhibitors to improve glucose control in T2-DM, has led to the examination of natural products with DPPIV inhibitory properties (Mardanyan et al., 2011). This study has shown that a mixture of flavonoids from *A. cominia* extracts inhibited the enzymatic activity of DPPIV *in vitro*. Though extracts of *A. cominia* have earlier been shown to have this property (Sánchez et al., 2014), the specific compounds or class of compounds were not identified or characterised. No results were shown regarding quercitrin and mearnsitrin separately as these two compounds (extracted from *A. cominia*) did not show any effect on the enzymes assays. However, they were only active as a mixture. The reason could be that these compounds might work only in a synergistic manner as when tested separately, the compounds did not show activity. The flavonoid rich fraction containing mixtures of several flavonoids produced a concentration dependent inhibition of DPPIV enzyme with a K_i three times higher than that of P32/98 inhibitor. The flavonoid mixture and the pheophytins produced a concentration dependent inhibition of PTP1B enzyme with K_i values of 3.00 μ g/ml for the flavonoids, 0.64 μ g/ml for pheophytin A and 0.88 μ g/ml for pheophytin B. These low K_i values reflect the high potencies of these compounds which confirm the assay results. Kinetic studies demonstrated that the inhibition produced by the flavonoid mixture and pheophytin A as well as the TFMS inhibitor of PTP1B enzyme indicates they were competitive inhibitors. Such compounds from plants including phenols, flavonoids and other compounds have been reported as PTP1B inhibitors (Zhang et al., 2010; Na et al., 2006a, b). These reports show that PTP1B enzyme inhibiting compounds from plants belong to diverse chemical classes. Only samples containing flavonoid components inhibited α -glucosidase enzyme. The flavonoid mixture produced a concentration dependent inhibition of α -glucosidase with a K_i value of 2.00 μ g/ml. The activity of the flavonoids was comparable to that of the glucosidase inhibitor (Acarbose) used by patients with T2-DM. Therefore, natural α -glucosidase inhibitors from *A. cominia* could be attractive for the management of postprandial hyperglycaemia (Koch and Deo, 2016). Other studies have isolated potential α -glucosidase inhibitors from plant extracts e.g. cranberry, pepper and soybean extracts (Apostolidis et al., 2006; Pullela et al., 2006; and Georgetti et al., 2006). Inhibition of α -glucosidase enzyme, by *A. cominia* extracts can be predicated on the decrease of glucose levels in the blood and could play important roles in the management of T2-DM (Ali et al., 2006; Bhat et al., 2008; Ye et al., 2002; and Zhong et al., 2006).

The present study identified the major compounds responsible for the relevant biological activities tested on the therapeutic targets associated with metabolic disorders such as T2-DM and obesity. Furthermore this research

complements previous results evaluating *A. cominia* total aqueous extract and its fractions on PTP1B and DPPIV (Sánchez et al., 2013) and contributes to explain some mechanisms involved in the hypoglycemic effect observed on *in vivo* on type 2 diabetic rat models by *A. cominia* aqueous extracts (Sánchez et al., 2014).

Mechanisms that have a high impact on glucose uptake are numerous, such as activation of glucose transporters GLUT4 in cell plasma membranes, insulin mimetic acting at post-receptor level, activation of AMP Kinase, an increase in nitric oxide, glucose-6P, glycogen stores (Wiernsperger, 2005) and deficiency in PTP1B. Further, many plants extracts may inhibit the action of the enzyme PTP1B that regulates negatively the insulin signalling by dephosphorylating phosphotyrosine residues on the insulin receptor. Plants, such as *Galega officinalis*, can also stimulate the glucose uptake (Mooney et al., 2008). Wiernsperger (2005) found that the inhibition of PTP1B is the main principle underlying the type of approach according to the activation of glucose uptake.

Prolonged DPPIV inhibition has a potential effect on reducing post-prandial glucose levels and HbA1c levels, which confirms that DPPIV inhibitors are novel, efficient and tolerable treatment of T2-DM (Ahrén, 2005).

α -Amylase and α -glucosidase inhibitors such as acarbose act by blocking the activity of these enzymes leading to a reduction in the breakdown of polysaccharides, and thereby decreasing the post-prandial increase in the blood glucose level in addition inducing weight loss (Zhong et al., 2006). Therefore, further assays were undertaken and results confirming the mechanism of action of these compounds will be proven. Nevertheless in the present paper the extract AE from *A.cominia* showed inhibition of the enzymatic activity of those relevant therapeutics targets of type 2 diabetes and obesity.

Funding

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5Uncited references

(Véliz et al., 2003; Ye et al., 2002).

Acknowledgements

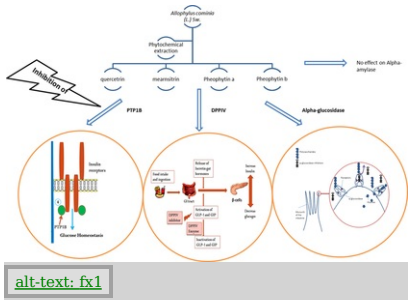
Many thanks to Dr Sc. Eva Marrero from CENSA, Cuba for holding and assisting with the plant material as part of the research team.

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